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Tubular Fluid Secretion in the Seminiferous Epithelium: Ion Transporters and Aquaporins in Sertoli Cells

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Abstract Sertoli cells play a key role in the establishment of an adequate luminal environment in the seminiferous tubules of the male reproductive tract. Secretion of the seminiferous tubular fluid (STF) is vital for the normal occurrence of spermatogenesis and for providing a means of transport to the developing spermatozoa. However, several studies on this subject have not completely clarified the origin and composition of this fluid. Electrolyte and water are central components of STF. Sertoli cells secrete an iso-osmotic fluid with a higher content of K^+ than the blood and express various membrane and water transporters (Na^+/K^+-ATPase; Ca^{2+}-ATPase; V-type ATPase; Cl^- channels; CFTR Cl^- channels; K^+ channels; L-, T- and N-type Ca^{2+} channels; Na^+/H^+ exchangers; Na^+-driven HCO_3^-/Cl^- exchangers (NDCBEs); Na^+/HCO_3^- cotransporters (NBCes); Na^+–K^+–$2Cl^-$ cotransporter; Na^+/Ca^{2+} exchanger; and aquaporins 0 and 8) involved in cellular and secretory functions. Studies with knockout mice for some of these transporters showed tubular fluid accumulation and associated infertility, revealing the relevance of these processes for the normal occurrence of spermatogenesis. Nevertheless, the role of the several membrane transporters in the establishment of STF electrolyte composition needs to be further elucidated. This review summarizes the available data on the ionic composition of STF and on the Sertoli cell membrane mechanisms responsible for ion and water movement. Deepening the knowledge on the mechanisms involved in the secretion, composition and regulation of SFT is essential and will be a major step in understanding the infertility associated with some pathological conditions.

Keywords Sertoli cell · Membrane ion transporter · Aquaporin · Seminiferous tubular fluid · Spermatogenesis · Blood–testis barrier

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Introduction

Testes are responsible for two major tasks: testosterone production and formation of haploid germ cells. These functions are primarily regulated by pituitary gonadotropins, with luteinizing hormone (LH) acting on the testosterone-producing Leydig cells located in the interstitium and follicle-stimulating hormone (FSH) acting on Sertoli cells in the seminiferous tubules (Griswold 1998; Walker and Cheng 2005). Nevertheless, a chain of complex local interactions involving the various testicular cell types, such as germ, Sertoli, peritubular and Leydig cells, are responsible for spermatogenesis control (Shubhada et al. 1993; Walker and Cheng 2005). Spermatogenesis is the process by which immature germ cells undergo division, differentiation and meiosis to give rise to haploid elongated spermatids. This process takes place within seminiferous tubules, the functional unities of the testis, through close association of germ cells with epithelial somatic cells, the Sertoli cells.

Sertoli cells play a central role in the development of functional testis and, consequently, in the expression of a male phenotype (Mruk and Cheng 2004; Sharpe et al. 2003). They influence testis formation in the embryo and spermatogenesis in the adult by regulating the surrounding environment of the developing germ cells (Griswold 1998).

Within the seminiferous tubules, the Sertoli cells reside on the basement membrane, under which are the lymphatic

endothelium and the peritubular myoid cells (Dym and Fawcett 1970). Sertoli cells face the lumen of the seminiferous tubule, providing structural support and creating an immunologically protected space for germ cells (Catalano et al. 2007). The structure of these cells is extremely complex, with numerous cup-shaped projections surrounding the various germ-cell types (Mruk and Cheng 2004; Russell 1993). Developing germ cells form intimate associations with Sertoli cells, and at a given moment various germ-cell types are in contact with one Sertoli cell. The various generations of germ cells are not randomly distributed within the seminiferous epithelium but are arranged in strictly defined cellular associations (Leblond and Clermont 1952; Mruk and Cheng 2004). The cell-to-cell interactions, not only between Sertoli cells and specific germ cells but also between Sertoli cells, are essential in the regulation of mammalian spermatogenesis (Jegou 1993; Mruk and Cheng 2004). These direct intercellular contacts are accomplished via the establishment of different types of junctions. Adherent, tight and gap junctions are implicated in cell adhesion (Lui et al. 2003a); the regulation of paracellular diffusion; the establishment and maintenance of cell polarity (Lui et al. 2003b); and intercellular communication processes (through the establishment of intercellular plasma membrane coupling channels) (Pointis et al. 2010) that occur in the seminiferous epithelium. Germ-cell development relies on a highly coordinated interaction with Sertoli cells, and they can communicate directly via ligand/receptor-mediated interactions or paracrine factors (Forti et al. 1992; Mruk and Cheng 2004) and through intercellular channels, consisting of gap junctions and their constitutive proteins, the connexins (Pointis et al. 2010). The production and secretion of many Sertoli-cell proteins and factors involved in germ-cell development occur in a stage-dependent manner, reflecting the ability of the Sertoli cell to adapt to the changing needs of the germ cell (Mruk and Cheng 2004).

Without the physical and metabolic support of the Sertoli cells, germ-cell differentiation, meiosis and transformation into spermatozoa will not occur (Lui et al. 2003b; Sharpe 1994). The number of Sertoli cells determines the number of germ cells that can be supported through spermatogenesis and, hence, numerically determines the extent of sperm production since each Sertoli cell has a fixed capacity for the number of germ cells that it can support (Petersen and Soder 2006; Sharpe 1994). Hence, a well-functioning Sertoli cell provides the developing germ cells with the appropriate nutrients, energy sources, hormones and growth factors and protects them from harmful agents and from the host's own immune system (Petersen and Soder 2006). Sertoli cells also control the composition of the seminiferous tubular fluid (STF), the physicochemical milieu where spermatogenesis occurs.

Seminiferous Tubular Fluid

The two-compartment configuration of the testis (the seminiferous tubules and the intertubular areas), filled with the characteristic fluids (STF and testicular lymph or interstitial fluid) (Jegou et al. 1982), is of great relevance for its functioning (Fig. 1).

It has been shown that the composition of the fluid within the seminiferous tubules is very stable due to the existence of the blood–testis barrier (BTB). The BTB regulates the passage of various endogenous and administered substances (Koskimies and Kormano 1973), supporting the fundamental relevance of the intraluminal fluid composition (Fisher 2002; Pace et al. 2000; Setchell 1980). It provides the milieu for developing spermatozoa and the vehicle to transport them from the testis. The luminal milieu is markedly distinct from the interstitial fluid and plasma and is critical to the occurrence of spermatogenesis (Fisher 2002; Pace et al. 2000). This hypothesis has long been supported by the observation that meiosis can be completed only after fluid secretion has been established (Setchell 1970), by the difficulties in accomplishing spermatogenesis in vitro despite the fact that there has been much effort to develop such cell culture systems (Kierszenbaum 1994; Steinberger 1975; Tesarik et al. 1998) and, furthermore, by the fact that blocking the lumen of a single seminiferous tubule causes the failure of spermatogenesis in that tubule (Pilsworth et al. 1981).

The secretion of STF commences during sexual maturation, after formation of the BTB, and is dependent on FSH (Jegou et al. 1982, 1983). Sertoli cells regulate,

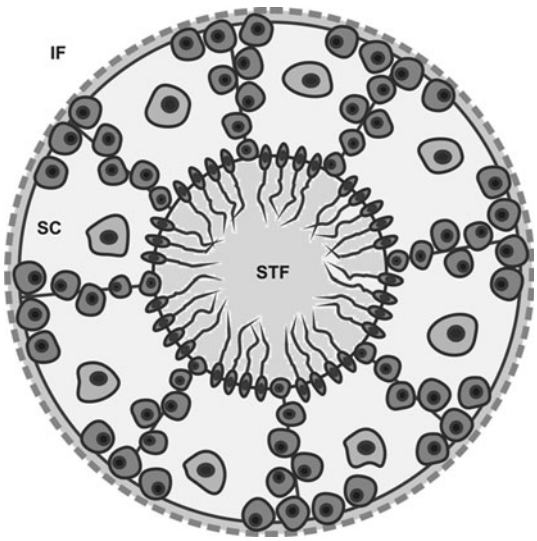

Fig. 1 The building unity of the blood–testis barrier. Simplified schematic representation of a cross section of a seminiferous tubule in a mammalian testis. *STF* seminiferous tubular fluid, *IF* interstitial fluid, *SC* Sertoli cell

among other things, the passage of ions and the selective flow of water, steroids and carbohydrates into the tubular lumen (Setchell and Waites 1975). The establishment of the ionic composition of the STF includes the net movements of water; Na^+, Cl^- and HCO_3^- reabsorption; K^+ secretion; and luminal acidification (Au and Wong 1980; Levine and Marsh 1971; Turner 1984).

Tuck et al. (1970) were the first to report the formation and ionic composition of the STF, and they postulated that Sertoli cells were responsible for fluid secretion in seminiferous tubules. However, until now, studies have not been able to satisfactorily clarify the origin and composition of this fluid (Fisher 2002; Setchell 1978; Waites and Gladwell 1982). A number of difficulties exist in developing a model that integrates all previous findings in this area. The proposed composition of fluid collected from the tubule varies depending on the method of collection (Fisher 2002; Henning and Young 1971; Levine and Marsh 1975; Tuck et al. 1970).

Substantial differences in the ionic composition of STF have been reported, especially in the concentration of K^+ but also in that of Na^+, Cl^- and HCO_3^-. Tuck et al. (1970), using a variation of the micropuncture technique, found that the fluid, which they called the "primary fluid," contained a high K^+ concentration but low Na^+ and Cl^- concentrations (Na^+ 38, K^+ 112, Cl^- 62 mM). The HCO_3^- concentration was estimated to be approximately 88 mM. These values were significantly different from another fluid (Na^+ 108, K^+ 45, Cl^- 120 mM), called the "free-flow fluid," which they collected spontaneously from the tubule without prior oil injection. Based on their findings, Tuck et al. (1970) suggested that the seminiferous epithelium secretes a K^+-rich solution which would be mixed in the tubule with an Na^+-rich/low K^+-containing fluid secreted by the rete testis.

The electrolyte composition of STF was also studied by Jenkins et al. (1980). The concentrations of Na^+, K^+ and Cl^- in rat STF were in agreement with those previously found (Jenkins et al. 1980; Levine and Marsh 1971; Tuck et al. 1970). The mean concentrations for Na^+ and K^+ in normal rat STF were 135.44 and 39.77 mM, respectively. These values were almost identical to those reported by Muffly et al. (1985) in tubules of Sertoli cell–enriched testis, leading the authors to conclude that Sertoli cells were responsible for the high concentration of K^+ in the STF, the most striking feature of the composition of this fluid. Na^+, Cl^- and Ca^{2+} concentrations in the fluid were not significantly different from those in serum and Mg^{2+} concentration was three times higher (Jenkins et al. 1980).

Later, it was shown, through microscopic examination and alternative nonperfusion electrophysiological measurements of the electric potentials, that perfusion techniques damaged the rat seminiferous epithelium (Fisher 2002). These results revealed that the seminiferous epithelium is very fragile and that its integrity is vulnerable to mechanical insults associated with experimental manipulations. Fisher (2002) believed, although without confirming its composition, that the free-flow samples are representative of the secreted intratubular fluid and that the seminiferous tubule is solely responsible for the production of its luminal fluid, opposing the previous "Tuck hypothesis."

More recently, Clulow and Jones (2004) reported consistent contaminations of the intratubular seminiferous fluid with intracellular solute, particularly K^+ and protein, when using the micropuncture technique and determined that the most suitable approach for defining the composition of the secretions of the seminiferous epithelium is to examine frozen sections by X-ray microanalysis. Measurements of the major electrolytes in Japanese quail STF showed a fluid rich in Na^+ and Cl^- content, with a K^+ concentration at least twice that of the blood (Na^+ 179, K^+ 13, Cl^- 170 mM), and indicated that this fluid is the main source of the luminal solutes in the extratesticular ducts.

Membrane Ion Transport in Sertoli Cells

Studies on the nature and role of the membrane transporters involved in Sertoli cell ion regulation are scarce and sometimes contradictory. Even so, several reports have shown that Sertoli cells express various types of ion membrane transporters (Table 1).

The presence of Na^+/K^+-ATPase on the adluminal portion of the plasma membrane of Sertoli cells, which pumps K^+ into the luminal fluid, was initially suggested by Hinton and Setchell (1993) and Byers and Graham (1990) by immunodetection on rat seminiferous tubules. Na^+/K^+-ATPase, or the sodium pump, uses the energy of one molecule of ATP to drive three Na^+ ions out of the cell and two K^+ ions into the cell against their concentration gradients. The main basic function of this pump is to maintain the Na^+ and K^+ gradients across the plasma membrane. In the model proposed by those authors (Byers and Graham 1990; Hinton and Setchell 1993), Na^+ and K^+ move across the adluminal surface of the membrane of Sertoli cells over a concentration gradient through this pump, which is responsible for the maintenance of Na^+ and K^+ balance.

The activity of this ion pump energizes diverse functions, such as maintenance of the membrane potential and of nutrient and ion compositions of several body fluids, and is responsible for fluid movement across transport epithelia (Lingrel 1992). It provides the energy for Na^+-coupled transport of various nutrients into the cell including glucose, amino acids and vitamins. The movement of ions such as Ca^{2+} and H^+ across the membrane as well as the maintenance of osmotic balance and cell volume also depend on the Na^+ and K^+ gradient.

Table 1 Ion membrane transporters described on the plasmatic membrane of Sertoli cells

Type	Identification method	References
Ion pumps		
Na^+/K^+-ATPase	IM	Hinton and Setchell (1993), Byers and Graham (1990)
Ca^{2+}-ATPase	EM	Feng et al. (2006)
H^+-ATPase	FM	Oliveira et al. (2009a, b)
Ion transporters		
NKCC	NA, FM	Pace et al. (2000)
NBC	FM	Oliveira et al. (2009a, b)
NDCBE	FM, NA	Oliveira et al. (2009a, b), Boron (2001)
NCX	RLA	Grasso et al. (1991)
NHE	FM	Oliveira et al. (2009a, b)
Ion channels		
L-type Ca^{2+} channel	IM	Taranta et al. (1997)
N-type Ca^{2+} channel	IM	Taranta et al. (1997)
T-type Ca^{2+} channel	PC	Lalevee et al. (1997)
P/Q-type Ca^{2+} channel	FM	D'Agostino et al. (1992)
Cl^- channel	RT-PCR, IM	Auzanneau et al. (2003), Babenko et al. (1998)
CFTR Cl^- channel	RT-PCR, PC	Boockfor et al. (1998)
K^+ channel	MT	Von Ledebur et al. (2002), Loss et al. (2004), Wassermann and Loss (2004)
Water channels		
AQP0	IM	Hermo et al. (2004)
AQP8	IM	Badran and Hermo (2002), Tani et al. (2001)

AQP aquaporin, *CFTR* cystic fibrosis transmembrane conductance regulator, *EM* electron microscopy, *FM* fluorescence microscopy, *IM* immunodetection, *MT* microelectrode techniques, *NA* Northern blot analysis, *NBC* Na^+/HCO_3^- cotransporter, *NDCBE* Na^+-driven HCO_3^-/Cl^- exchanger, *NCX* Na^+/Ca^{2+} exchanger, *NHE* Na^+/H^+ exchanger, *NKCC* Na^+–K^+–$2Cl^-$ cotransporter, *PC* patch-clamp technique, *RLA* radiolabeled ion-flux assay

In Sertoli cells, a number of membrane transport systems that depend on this Na^+ gradient generated by Na^+/K^+-ATPase have been identified. For instance, Na^+-driven membrane transporters of HCO_3^- and H^+ ions in Sertoli cells have been described by Oliveira et al. (2009a, b), using human and bovine primary cultures and specific inhibitors.

Those authors suggested the presence of Na^+-driven HCO_3^-/Cl^- exchanger (NDCBE) and Na^+/H^+ exchanger, and of Na^+/HCO_3^- cotransporters (NBCes), all of which have been classified as acid extruders (Boron 2001). They mediate the exit of H^+ from the cell or the uptake of HCO_3^- (Boron 2004). In epithelial cells, the above acid-base transporters show distinct membrane-domain localization, with certain transporters demonstrating exclusive expression in either the luminal or basolateral membrane, which results in vectorial transport of bicarbonate from lumen to blood or vice versa (Boron 2001; Soleimani and Burnham 2001).

The NDCBE and the NBCes are members of a family of ion membrane transporters known as Na^+-coupled HCO_3^- transporters (Boron 2001; Romero et al. 2004). Similar to what happens in other epithelia, in Sertoli cells these transporters must have a central role in the regulation of intracellular pH in cells, as well as in the transport of acid-base equivalents (and/or salt) across the seminiferous epithelia responsible for maintaining the slightly acidic pH of the lumen of the tubule (Caflisch and DuBose 1990; Levine and Marsh 1971).

The NDCBEs are electroneutral transporters, which means that two base equivalents are cotransported with sodium in exchange for chloride. This subfamily is constituted by at least four protein variants (NDCBEs A–D) that seem to be the major pHi regulators in many cells (Boron et al. 2009; Boron 2001; Parker et al. 2008). Besides Sertoli cells, NDCBEs are also highly expressed in the testis (Boron 2001). The NBCes consist of a subfamily of sodium-coupled bicarbonate transporters with two isoforms (NBCe1 and NBCe2), which operate with variable tissue-specific stoichiometries (Boron et al. 2009; Soleimani and Burnham 2001). These cotransporters are capable of mediating Na^+:$2HCO_3^-$ influx or Na^+:$3HCO_3^-$ efflux in a tissue-specific manner and are essentially involved in pHi regulation (Boron et al. 2009; Boron 2001).

The Na^+/H^+ exchangers (NHEs) are a family of membrane proteins that regulate the extrusion of one intracellular proton in exchange for one extracellular sodium ion. This family of ion exchangers consists of nine known isoforms (NHE1–NHE9) involved in a variety of complex physiological and pathological events that include regulation of intracellular pH and cell movement (Malo and Fliegel 2006). Among those isoforms, NHE1, the first to be

identified in freshly isolated Sertoli cells (Gorczyńska-Fjälling 2004), is ubiquitously expressed in all mammalian cells and plays a "housekeeping role" (Malo and Fliegel 2006). In addition, NHE2 and NHE3 are apical membrane proteins that are known to be expressed in the testis and in freshly isolated Sertoli cells (Gorczyńska-Fjälling 2004; Malakooti et al. 1999; Zhou et al. 2001). Even if the function of NHE3 on the seminiferous tubules has not been elucidated, it has been shown that this transporter is important to the overall function of the testis. NHE3-knockout mice exhibit tubular fluid accumulation associated with infertility (Zhou et al. 2001). These results demonstrated the relevance of NHE3 in tubular fluid secretion and the role of the expression of this membrane transporter in the physiology of the male reproductive tract. Nevertheless, it should not be overlooked that some of these transporters are also present on the plasma membrane of developing spermatogenic cells (Wang et al. 2003, 2007) and that its malfunction could also be associated with the reproductive disorders and with the abnormal tubular fluid secretion reported.

Na^+–K^+–$2Cl^-$ cotransporters are also among the Na^+-dependent membrane transporters that have been identified in freshly isolated mouse Sertoli cells (Pace et al. 2000). These proteins are expressed in a broad spectrum of tissues and implicated in cell volume regulation and in ion transport by secretory epithelial tissue (O'Grady et al. 1987; Russell 2000), namely in the seminiferous epithelium (Pace et al. 2000). Molecular biology studies identified two isoforms of the Na^+–K^+–$2Cl^-$ cotransporters, NKCC1 and NKCC2. mRNA analysis showed that NKCC1 is expressed in the various cells of the male reproductive tract (Delpire et al. 1994; Pace et al. 2000). Although its direct contribution to fertility has not been disclosed, NKCC1-deficient mice presented only inner ear defects and were infertile due to defective spermatogenesis (Pace et al. 2000). Given the tissue distribution of NKCC1 in the seminiferous epithelium and its proposed significance in epithelia, the observation of a severe phenotype only in testis (and inner ear) was described as a sign of the critical role for NKCC1-mediated ion transport in spermatogenesis (Pace et al. 2000).

The sodium–calcium exchanger (NCX), which transports Na^+ and Ca^{2+}, is a ubiquitously expressed membrane protein essential in calcium homeostasis (Blaustein and Lederer 1999). NCX can move Ca^{2+} either into or out of cells, depending on the net electrochemical driving force on the exchanger. The net Ca^{2+} movement mediated by the exchanger may change direction during a cell's activity cycle, when the membrane potential varies and/or when the cytosolic Na^{2+} or Ca^{2+} concentration is altered (Philipson and Nicoll 2000; Schulze et al. 2003). This exchanger activity is regulated by these substrates (Na^{2+} and Ca^{2+}) but also by protons, ATP and diverse other modulators. NCX expression has been described in isolated Sertoli cells, where calcium levels depend directly on sodium fluxes, similar to what happens in other systems with this ion exchanger (Grasso et al. 1991). Furthermore, it has been shown that NCX is also involved in the transmembrane sodium flux in isolated FSH-stimulated rat Sertoli cells (Gorczynska and Handelsman 1991, 1993; Grasso et al. 1991).

Various types of ion channels have also been identified in Sertoli cells. Ion channels function as pores for the passive diffusion of ions across biological membranes. They are selective for a particular ionic species (or a restricted group of ions), and the direction of the net ion transport depends on the established electrochemical gradient. Ion channels can close and open, which allows regulation of these transporters. There are ligand-gated, voltage-gated, swelling- or stretch-activated, and heat- or cold-activated channels. In addition, channels may be regulated by Ca^{2+}, pH, phosphorylation and lipids (Hubner and Jentsch 2002). Ion channels have numerous functions, from transduction of electrical and chemical signaling to transepithelial transport, regulation of cell volume and regulation of cytoplasmic or vesicular ion concentration and pH (Hubner and Jentsch 2002).

Using cultured rat Sertoli cell epithelia, Ko et al. (1998) showed that apical ATP stimulates a short-circuit current (Isc). The ATP-induced Isc was not sensitive to various pharmacological agents but only to apical diphenylamine-2-carboxylate (DPC) or 4,4,-disothiocyano-2,2,disulfonic acid stilbene (DIDS), blockers of Cl^- channels. Removal of extracellular HCO_3^- also had little effect on the simulated Isc, whereas removal of Cl^- abolished the Isc response. Sertoli cells exhibited a DIDS-sensitive outwardly rectifying Cl^- conductance with voltage dependence (Babenko et al. 1998). Moreover, expression of several isoforms of voltage-dependent Cl^- channels was described in rat Sertoli cells as being responsible for a Cl^- current that was activated by acidic extracellular pH (pHe) (Auzanneau et al. 2003). The family of voltage-dependent chloride channels (ClC) is composed of nine members in mammalian cells (Jentsch et al. 2002). It has been shown that rat Sertoli cells express ClC-1, ClC-2, ClC-3, ClC-6 and ClC-7 isoforms (Auzanneau et al. 2003; Menegaz et al. 2010).

It was also reported that the acid-sensing Cl^- channel is regulated by the endogenous transient receptor potential vanilloid 1 (TRPV1) (Auzanneau et al. 2008). TRPV1 is a transient receptor potential channel that works as a biological sensor, detecting changes in the environment (Minke 2006). It is characterized by permeability to several cations, including Na^+ and Ca^{2+}, and was first described in Sertoli cells by Rossi et al. (2007). TRPV1 is localized in the plasma membrane and cytosol of Sertoli cells, and it has been suggested to regulate the acid-sensing Cl^- channel (Auzanneau et al. 2008; Rossi et al. 2007).

Secretory activities of Sertoli cells are critical to spermatogenesis (Griswold 1998), and voltage-gated Cl^- channels function frequently as an electric shunt that couples to H^+-ATPase-driven loading of secretory vesicles and are, therefore, crucial for the onset of exocytosis (Decoursey 2003; Jentsch et al. 2002). Supporting this idea, and although Herak-Kramberger et al. (2001) described that seminiferous tubules were immunonegative for vacuolar H^+-ATPase, Oliveira et al. (2009a, b) recently reported the activity of H^+-ATPases in human and bovine Sertoli cells. These authors pointed to a negligible participation of H^+-ATPase on pHi regulation mechanisms, suggesting that it may be primarily involved in cellular secretory phenomena.

K^+ channels have likewise been identified in the plasma membrane of these cells (Von Ledebur et al. 2002). Results using specific inhibitors for these channels have demonstrated the action of sex hormones on the membrane potential of Sertoli cells in rat seminiferous tubules, using intracellular microelectrode techniques (Loss et al. 2004; Von Ledebur et al. 2002; Wassermann and Loss 2004). Adenosine triphosphate–sensitive K^+ (K^+_{ATP}) is usually inhibited by increasing intracellular ATP and open during periods of energy depletion (Babenko et al. 1998). In endocrine cells, K^+_{ATP} channels exert a role also on hormone signaling through membrane depolarization and Ca^{2+} influx (Dunne and Petersen 1991; Wassermann and Loss 2004). In Sertoli cells, the rapid action of testosterone seems to be associated with the closing of these channels and with the resulting depolarization of the plasmatic membrane (Jacobus et al. 2005; Loss et al. 2004; Von Ledebur et al. 2002). Although it has not yet been described, we cannot overlook the hypothesis that these or other types of K^+ membrane channels contribute to the epithelial transport of potassium toward the lumen of the seminiferous tubules, where the concentration of this ion seems to be at least double that in the plasma (Clulow and Jones 2004).

The presence of four types of calcium channels (L-, N-, P/Q- and T-type voltage-gated Ca^{2+} channels) was equally identified in the membrane of rat Sertoli cells using different techniques (D'Agostino et al. 1992; Lalevee et al. 1997; Taranta et al. 1997). Gorczynska and Handelsman (1991) exposed Sertoli cells to increasing doses of FSH and demonstrated a dose-dependent increase of cytosolic Ca^{2+} concentration. It was suggested that the activation of Ca^{2+} flux in freshly isolated Sertoli cells through one or more of these types of Ca^{2+} channels is mainly involved in the hormone-induced rise of cytosolic Ca^{2+} (Gorczynska and Handelsman 1991) and not directly involved in the establishment of the STF composition.

Another type of ion channel expressed in primary cultures of rat Sertoli cells is the cystic fibrosis transmembrane regulator (CFTR). Boockfor et al. (1998) showed, using a patch-clamp approach, that Sertoli cells express a Cl^- channel consistent with the characteristics of CFTR. Stimulation of cAMP-dependent protein kinase on Sertoli cells resulted in the activation of a time-independent Cl^- current. CFTR is believed to function as a cAMP-dependent, protein kinase-activated Cl^- channel (Bear et al. 1992). In other tissues, such as the lung, pancreas and intestine, the CFTR Cl^- channel plays an important role in fluid and electrolyte transport (Riordan 1993), so it is presumable that CFTR in Sertoli cells may also have a relevant contribution to those events.

Finally, in addition to the ion pumps describe above (Na^+/K^+-ATPase and H^+-ATPase), Ca^{2+}-ATPase has been described by cytochemical techniques in the plasma membrane of the Sertoli cells in hamster seminiferous tubules (Feng et al. 2006). The plasma membrane Ca^{2+}-ATPase (PMCA) is a transport protein that can extrude Ca^{2+} against large concentration gradients. It is prominently involved in the regulation of intracellular free Ca^{2+} in all eukaryotic cells (Carafoli 1992). Furthermore, PMCAs also function in some epithelia in order to regulate extracellular Ca^{2+} concentration (Talarico et al. 2005). Nevertheless, Feng et al. (2006) associated the presence of Ca^{2+}-ATPases in the plasma membrane of Sertoli cells only with the maintenance of a low intracellular Ca^{2+} concentration, by pumping Ca^{2+} out of the cell.

Water Membrane Transport in Sertoli Cells

Water movements are also essential for creating the fluid environment for the progression of sperm toward the rete testis and to the epididymis (Hinton and Setchell 1993; Voglmayr et al. 1970).

In mammalian cells, water can cross cell membranes by simple diffusion across the lipidic bilayer or by bulk flow (driven by an osmotic gradient) through hydrophilic pores. The water-channel proteins that are responsible for the movement of water across membranes are generally named aquaporins (AQPs) (Agre et al. 2002; Preston and Agre 1991; Verkman and Mitra 2000). These water channels are essential for the regulation of water homeostasis and for providing a continuous and rapid movement of water across tight junction epithelia (Agre et al. 2002; Brown et al. 1993; Wintour 1997). So far, 13 aquaporin subtypes (AQP0–12) have been described in mammalian cells (Ishikawa et al. 2006).

The expression of various AQPs has been described in germ cells and other testicular cell types (Badran and Hermo 2002; Hermo et al. 2004; Huang et al. 2006; Sohara et al. 2009; Yeung et al. 2010) and is consistent with the occurrence of fluid movement in testis. Furthermore, altered expression and regulation of AQPs have already

been demonstrated to be the cause of several disorders of the male reproductive system (Huang et al. 2006). Some AQP isoforms have been described in Sertoli cells (Badran and Hermo 2002; Hermo et al. 2004; Tani et al. 2001) and are most probably involved in the secretion of STF.

AQP8, proposed as an important water transporter in several tissues, was identified in rat Sertoli cells of immunostained frozen testicular tissue sections (Badran and Hermo 2002; Tani et al. 2001). Higher AQP8 expression in salivary gland, liver, pancreas, small intestine, colon and testis and lower expression in kidney and heart have been described (Elkjar et al. 2001; Ma et al. 1997; Yang et al. 2005). Yang et al. (2006) proposed that in mice AQP8 facilitates water and NH_3 transport, although the absolute contribution of AQP8 to NH_3 transport is smaller than that to water transport. That being so, AQP8 should not be regarded as a conventional AQP (Rojek et al. 2008). In rat seminiferous tubules AQP8 expression was localized exclusively in the Sertoli cells (Badran and Hermo 2002). AQP8 mRNA was found to be present homogeneously in every seminiferous tubule, which is consistent with the expression of AQP8 at the protein level (Elkjar et al. 2001). In contrast to the abundance of AQP8 in rat testis, AQP8 mRNA was absent in human testis (Koyama et al. 1998).

AQP8 in Sertoli cells would be involved in the transport of water from the interstitial space into the lumen. This appears to occur along an osmotic gradient, with the Na^+/K^+ pump already being described in Sertoli cells also in immunostained testicular tissue sections (Gravis et al. 1976). It is significant that AQP8 expression is localized on the apicolateral portion of the Sertoli cell plasma membrane, where the pumps are also located (Badran and Hermo 2002).

Surprisingly, a phenotypic analysis of AQP8 null mice (−/−) showed few and only mild phenotype differences between wild-type and AQP8-deficient (−/+) mice. Although testis weight and size in AQP8-null mice were increased, no impaired fertility or abnormalities in sperm count or morphology were found (Yang et al. 2005).

In rat, AQP0 expression in the seminiferous epithelium was also restricted to Sertoli cells (Hermo et al. 2004). AQP0, also known as the major intrinsic protein, is a water-permeable channel abundant in the ocular lens (Ball et al. 2003). It was the first member of the AQP family to be sequenced (Ball et al. 2003; Gorin et al. 1984), and it seems to confer water permeability to membranes, contributing to water homeostasis and to the circulation of nutrients within cells (Ball et al. 2003).

The expression of AQP0 in Sertoli cells presents a cyclic variation consistent with the progression of stages in the spermatogenic cycle (Hermo et al. 2004). It is specific to certain stages of the spermatogenic cycle, in contrast to the distribution noted for AQP8, which is expressed in all Sertoli cells of different tubules at the same time. AQP0 expression in Sertoli cells is more intense during stages 6–8, presenting moderate expression in stages 1–5 and weak expression in stages 9–14 (Hermo et al. 2004). The period of stage 6 corresponds to the moment prior to the elongating spermatids being released from the tubule, and stage 8 corresponds to its final release into the lumen of the tubule (Russell et al. 2008).

That being so, in Sertoli cells, AQP8 would be involved in the transport of water from the interstitial space into the lumen, and this would occur at all stages of the spermatogenic cycle. The finding that AQP0 in these cells is maximally expressed at stages 6–8 suggests that this water channel could also assist AQP8 in its function. Thus, the presence of two AQPs at this time point during the cycle may greatly facilitate the transport of water into the lumen and, hence, the movement of the germ cells out of the seminiferous tubules.

Conclusion

The generation of competent sperm is a complex multistep process that initiates in the seminiferous epithelium. One critical feature is the secretion of STF, which is known to be maintained slightly acidic (Caflisch and DuBose 1990; Levine and Marsh 1971). Most of the knowledge of the ionic composition of this luminal fluid comes from the work of independent groups (Clulow and Jones 2004; Fisher 2002; Henning and Young 1971; Setchell 1978; Tuck et al. 1970; Waites and Gladwell 1982), which described divergent results according to the methods of analysis used. Nevertheless, all groups agreed that transepithelial ion and water transport through the BTB is achieved mainly by the intervention of Sertoli cells. In these cells, distinct types of transport proteins have been identified, such as membrane pumps (Na^+/K^+-ATPase, Ca^{2+}-ATPase and a probable V-type ATPase), various H^+/HCO_3^- membrane transporters (NDCBEs, NBCes and Na^+/H^+ exchangers), ion channels (voltage-dependent Cl^- channels activated by acidic pHe, CFTR Cl^- channels, K^+ channels and L- T- and N-type Ca^{2+} channels), ion cotransporters (Na^+–K^+–$2Cl^-$ cotransporter and Na^+/Ca^{2+} exchanger) and water channels (AQPs 0 and 8). Although the involvement of such transporters in the establishment of the STF is not yet completely disclosed, it is certain that they have a key role in the cellular mechanisms responsible for determining ion composition, osmolarity and pH of the fluid.

Further knowledge of their functioning and regulation is essential for the enlightenment of a process that is central to the establishment of a suitable environment in

which germ cells mature to become functional spermatozoa. This will be an important step in understanding infertility linked to Sertoli-cell dysfunction and the role of these water and ion membrane transporters in some pathological conditions.

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